SUPPLEMENTAL FIGURE LEGENDS

Figure S1. OP449 in combination with ABL1 tyrosine kinase inhibitors inhibits BCR-ABL1 signaling in CML cells and cellular growth of primary CML blastic phase cells ex vivo. (A) Combined effects of OP449 and ABL1 kinase inhibitors on BCR-ABL1 signaling. K562 cells were cultured for 16 hrs in the presence of the indicated concentrations of inhibitors, and whole cell lysates were subjected to SDS-PAGE and immunoblotted using the indicated antibodies. **(B,C,D)** Combined effects of OP449 and ponatinib on proliferation of CML cells. K562 cells (B), ponatinib-sensitive sample (CML-02, C) and ponatinib-resistant sample (CML-08, D) were cultured in the presence of OP449 alone or in combination with ponatinib, and viability was measured at 72 hrs by standard MTS assay (panel B and D) or by colony formation in methocult containing cytokines. **(E)** Efficacy of OP449 in primary blastic phase CML sample. CML patients in lymphoid blastic phase disease (L-BP) were cultured in the presence of OP449 alone or in combination with dasatinib and cell viability was measured at 72 hrs by standard MTS assay. In all cases bars represent the mean percent relative to untreated cells ± standard deviation. **(F)** Induction of apoptosis by OP449 in primary CML cells. Primary CD34+ CML cells were cultured in graded concentrations of OP449, and apoptosis was measured at 48 and 72 hrs. Results are graphed as the mean percent annexin-positive cells ± standard deviation.

Figure S2. **SET is overexpressed in primary AML cells and OP449 inhibits growth of primary AML patient cells. (A)** SET expression levels in AML cells lines tested. SET/ PP2Ac ratios were quantified from densitometric analysis of protein expression in

each of the indicated AML cell lines. **(B)** SET expression levels in primary AML samples and normal CD34+ cells tested. SET/PP2Ac ratios were quantified from densitometric analysis of protein expression in each of the indicated samples. Dashed lines represent mean SET/PP2Ac ratios for normal CD34+ and AML cells, respectively. Bar graph shows mean SET/PP2Ac ratios for normal CD34+ and AML cells ± standard error.

OP449 – A SET antagonist with in vitro and in vivo anti-tumor activity.

We recently reported peptide antagonists of SET that potently increased cellular PP2A activity (1-3). These peptides are derived from our initial peptide known as COG133 that comprises the receptor binding domain of apolipoprotein E protein from amino acids 133-149. COG133 was reported to have anti-inflammatory activity in multiple assay systems and to have in vivo activity in reducing the production of inflammatory cytokines (4-6). The selectivity of these peptides for SET was demonstrated by preparing a biotin labeled version of the peptide and incubating the biotin-labeled peptide with a cell lysate. Purification of peptide-bound proteins was performed with streptavidin agarose and the bound proteins were resolved with PAGE and stained to reveal that only one protein was bound to the peptide (1). Furthermore, we also reported that COG445, a derivative of this original peptide activated PP2A in the MDA-MB-231 breast cancer cell line (3) and another related peptide COG1410 increased PP2A activity in the brain of an intact mouse following subcutaneous injection (1).

Based on these activities, we prepared over 400 analogs of these peptides and have screened them for their ability to regulate signal transduction pathways that are dependent on PP2A. This led to the selection of a subgroup of active compounds for testing of their ability to inhibit cancer cell growth. We screened these compounds for cytotoxicity against primary human CLL cells and evaluated their effect on normal human B-cells in order to determine the safety window. Based on this screen, we initially identified COG445 as a lead. To further understand the mechanism of action, we demonstrated that COG445, through activation of PP2A, downregulated Akt phosphorylation in a dose dependent manner. We also confirmed that the dephosphorylation of Akt was mediated by PP2A because the dephosphorylating activity was counteracted by chemical inhibition of PP2A using the small molecule PP2A inhibitor okadaic acid (3). Unfortunately, we found that the disulfide bond was reduced *in vivo* so we created COG449 (known as OP449 after Oncotide Pharmaceuticals acquired the rights to this compound from Cognosci Inc.). OP449 has a stabilized linker between the two COG112 monomers, thereby creating a dimer that maintained the activity of COG445 while being stable *in vivo*. We found that OP449 was cytotoxic to CLL cells, with an ED50 of 100 nM, while the ED50 for normal B-cells was found to be nearly 2-log units higher > 10 μM (2). A selection of the structure activity relationship data for these compounds is given in Table 1. Notably, we have previously demonstrated that biotinylated-COG112 could be used to affinity purify SET from MDA-MB-231 breast cancer cells, which demonstrated that the OP449 monomer binds to SET in tumor cells (3).

Table 1: Cytotoxicity of COG compounds for CLL cells (2)

Compound	Sequence
COG133	LRVRLASHLRKLRKRLL
COG1410	AS(Aib)LRKL(Aib)KRLL*
COG112	RQIKIWFQNRRMKWKK-C-LRVRLASHLRKLRKRLL
COG445	RQIKIWFQNRRMKWKK-C-LRVRLASHLRKLRKRLL
(disulfide linked COG112)	|
	RQIKIWFQNRRMKWKK-C-LRVRLASHLRKLRKRLL
OP449 (previously COG449)	RQIKIWFQNRRMKWKK-C-LRVRLASHLRKLRKRLL
(BMOE** linked COG112)	<bmoe>
	RQIKIWFQNRRMKWKK-C-LRVRLASHLRKLRKRLL

* Aib = aminoisobutyric acid ** BMOE = bismaleimidoethane

1. Christensen DJ, Ohkubo N, Oddo J, Van Kanegan MJ, Neil J, Li F, et al. Apolipoprotein E and peptide mimetics modulate inflammation by binding the SET protein and activating protein phosphatase 2A. J Immunol. 2011;186:2535-42.
2. Christensen DJ, Chen Y, Oddo J, Matta KM, Neil J, Davis ED, et al. SET oncoprotein overexpression in B-cell chronic lymphocytic leukemia and non-Hodgkin lymphoma: a predictor of aggressive disease and a new treatment target. Blood. 2011;118:4150-8.
3. Switzer CH, Cheng RY, Vitek TM, Christensen DJ, Wink DA, Vitek MP. Targeting SET/I(2)PP2A oncoprotein functions as a multi-pathway strategy for cancer therapy. Oncogene. 2011;30:2504-13.
4. Li FQ, Sempowski GD, McKenna SE, Laskowitz DT, Colton CA, Vitek MP. Apolipoprotein E-derived peptides ameliorate clinical disability and inflammatory infiltrates into the spinal cord in a murine model of multiple sclerosis. J Pharmacol Exp Ther. 2006;318:956-65.
5. Laskowitz DT, Fillit H, Yeung N, Toku K, Vitek MP. Apolipoprotein E-derived peptides reduce CNS inflammation: implications for therapy of neurological disease. Acta Neurol Scand Suppl. 2006;185:15-20.
6. Lynch JR, Tang W, Wang H, Vitek MP, Bennett ER, Sullivan PM, et al. APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. J Biol Chem. 2003;278:48529-33.